# THE COMPLEX FORMED BETWEEN THE A AND B PROTEINS OF LACTOSE SYNTHETASE

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Lactose synthetase activity depends on two protein components designated A and B, which are separable by gel filtration [1]. The A protein is a galactosyl transferase carrying out the reaction [2] :

UDP-galactose + *N*-acetylglucosamine $\xrightarrow{Mn^{2+}}$ N-acetyllactosamine + UDP.

B, which is α-lactalbumin [3], modifies the reaction, so that for A in the presence of B the following reaction occurs [3] :

UDP-galactose + glucose $\xrightarrow{Mn^{2+}}$ lactose + UDP.

The lactose synthetase system is of interest in understanding how one protein (B) modifies the catalytic properties of another (A). Although there are some indications of an association between the proteins [4–7], there is little direct information on the complex formed between them.

The present communication is concerned with a study of the interaction between the A and B proteins, and gives a preliminary account of such investigations in the presence of the galactosyl acceptors *N*-acetylglucosamine and glucose. It shows clearly that a discrete entity is formed containing one molecule of A and one molecule of B

Both A and B proteins were isolated from milk. The purification of A essentially followed the method of Andrews [4]. By this procedure 25 mg of A protein could be isolated from 50 ℓ of bovine milk. The method of Jones [8] was used to assay the enzyme. Investigations were carried out in *N*-ethylmorpholine-HCl at 0.05 I made up to 0.5 I with KCl, and 4 mM in $MnCl_2$. The pH was adjusted to 7.4 with *N*-ethylmorpholine. The concentrations of *N*-acetylglucosamine and glucose used were 5 mM and 100 mM, respectively.

Sedimentation of the purified A protein was studied in the above buffer solution at 10° at a speed of 59,780 rpm. Sedimentation coefficients were corrected to standard conditions using data from *International Critical Tables*, and assuming a partial specific volume, $\bar{v}$, of 0.73. This value of $\bar{v}$ corresponds to that calculated for the composition of a-lactalbumin and for the A protein alone [5]. Sedimentation of the A protein showed a single schlieren peak with an $s_{20,w}$ of 3.0 S. In the presence of B together with 5 mM *N*-acetylglucosamine the $s_{20,w}$ was altered to 3.5 S for the main component. A slow component remaining at the meniscus was present in the latter experiments, corresponding to the excess B present.

Molecular weights were determined by the method of Van Holde and Baldwin [9] using 3 mm solution columns and schlieren optics. For the purified A protein the presence of aggregates was indicated by the discrepancy between the weight-average molecular weight ($M_w$) and the z-average molecular weight ($M_z$) calculated over the solution column [9]. However, the calculations for $M_w$ and $M_z$ showed that over a partial range of the solution column towards the meniscus the graphs were linear (fig. 1). The values were 42,000 for $M_w$ and 48,000 for $M_z$ which are near the previously reported estimates [4–7].

For investigation of the complex the following procedure was adopted. A concentrated solution of A with excess B was applied to a short column of Sephadex G-100, equilibrated and eluted with buffer containing B and either *N*-acetylglucosamine or

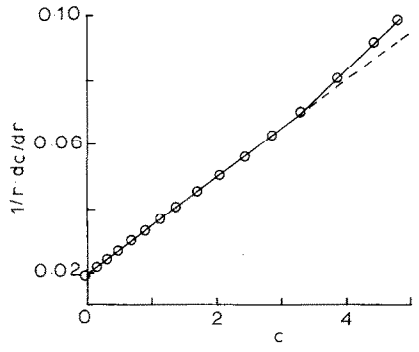


Fig. 1. The ratio of the refractive index increment to radial position plotted against concentration (in arbitrary units), giving $\bar{M}_z$ for the A protein.

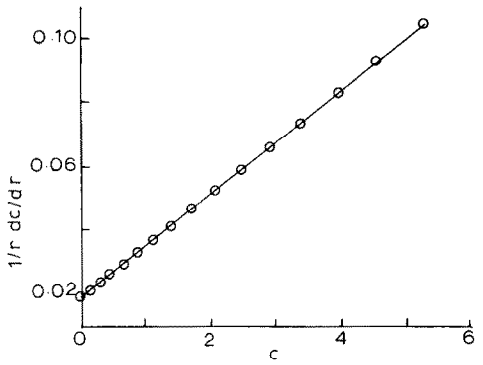


Fig. 2. The ratio of the refractive index increment to radial position plotted against concentration (in arbitrary units), giving $\bar{M}_z$ for the AB complex. The complex was in equilibrium with 3 mg/ml of B and 5 mM *N*-acetylglucosamine.

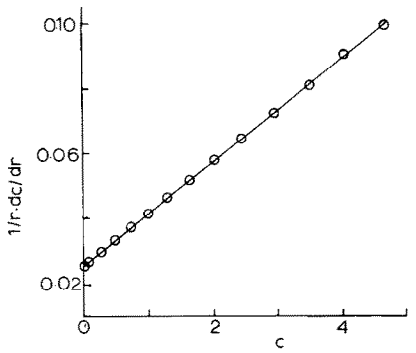


Fig. 3. The ratio of refractive index increment to radial position plotted against concentration (in arbitrary units), giving $\bar{M}_z$ for the AB complex. The complex was in equilibrium with 3 mg/ml of B and 100 mM glucose.

glucose at chosen concentrations. Any B in excess of that required for formation of the complex then appeared as a slow moving peak in the protein profile. After elution, fractions corresponding to the fast component were combined for analysis by equilibrium centrifugation. The reference solution was the elution buffer used in the gel-filtration. Since this provided a solution with which the complex was in equilibrium, the boundary-forming experiment should give an accurate measure of the concentration of the A protein and its complexes with B.

The results (table 1) show that the average molecular weights, $M_w$ and $M_z$, calculated from the extremes of the solution column are in good agreement for both galactosyl acceptors, and the ratio of $M_z$ to $M_w$ is of the order 1.02. The graphs of the data giving $\bar{M}_w$ and $\bar{M}_z$ are linear (fig. 2 and 3). The values obtained from the slopes of these lines with their standard errors are given in table 1 and show good agreement. The observed molecular weights are essentially constant in these experiments, namely with 3 mg/ml of B and either 5 mM *N*-acetylglucosamine or 100 mM glucose. Thus the weight-average and the z-average molecular weights are 58,500 and 59,500, respectively, which indicate a discrete homogeneous species with a molecular weight of 59,000 ± 500. The A protein has a minimum molecular weight near 45,000 from the reports of others [4–7] and also indicated in the present research. The molecular weight of B is 14,200 calculated from its amino acid composition [10]. It follows that the complex observed in the present experiments in the presence of *N*-acetylglucosamine or glucose consists of one molecule of each protein. The observed change in $s_{20,w}$ from 3.0 S for A to 3.5 S for AB is consistent with this finding since a value of 3.6 S would be expected for AB from the change in molecular weight assuming the proteins were of similar shape.

The molecular weight data suggests that under these experimental conditions a discrete species was observed. Allowing for possible experimental errors

Table 1

The molecular weight of the complex between the A and B proteins of lactose synthetase.

| Substrate present | $10^3 \times M_w$ | $10^3 \times M_z$ | $M_z/M_w$ | $10^3 \times \bar{M}_w$ | $10^3 \times \bar{M}_z$ | $10^3 \times \bar{M}_w(r)$ |
|---|---|---|---|---|---|---|
| NAG | 58.3 | 59.4 | 1.02 | 58.2 ± 0.1 | 59.5 ± 0.1 | 58 ± 1 |
| Glu | 58.2 | 59.3 | 1.02 | 58.1 ± 0.1 | 59.5 ± 0.1 | 58 ± 1 |

The values $M_w$ and $M_z$ were calculated from data at the extremes of the solution column [9]. $\bar{M}_w$ and $\bar{M}_z$ were calculated from appropriate plots of data through the solution column. $\bar{M}_w(r)$ is the mean of values calculated at various radial positions. All experiments were in the presence of 3 mg/ml of B, and 4 mM manganese chloride. The *N*-acetylglucosamine (NAG) concentration was 5 mM and the glucose (Glu) concentration was 100 mM.

it is therefore probable that at least 90% of the A protein is present as the complex. From work in this laboratory and others [4–7] it is apparent that complex formation depends on the presence of a galactosyl acceptor. Hence the association constant for formation of the ternary complex is

$$K_{NAG} = \frac{[A{-}NAG{-}B]}{[A]\,[NAG]\,[B]} \quad \text{and}$$

$$K_{Glu} = \frac{[A{-}Glu{-}B]}{[A]\,[Glu]\,[B]}\ .$$

With the above estimate for the proportion of A in the complex, it follows that the minimum values for the association constants are $1 \times 10^7$ ($\ell$/mole)$^2$ for $K_{NAG}$ and $5 \times 10^5$ ($\ell$/mole)$^2$ for $K_{Glu}$. These values suggest a reasonably tight binding between A, B and either *N*-acetylglucosamine or glucose.

The natural substrate for the A protein is a glycoprotein with terminal *N*-acetylglucosamine. The reaction is not influenced by the presence of B [11]. However, with free *N*-acetylglucosamine or glucose, the presence of B increases the binding of these substrates [12, 13]. The present work shows that there is tight binding in the ternary complex of A, B and either *N*-acetylglucosamine or glucose, which suggests that the interactions in the complex approximate to the interactions between A and a glycoprotein substrate. This suggests that B with either *N*-acetylglucosamine or glucose behaves as a glycoprotein analogue. Hence the apparent modifying effect of the B protein could arise from its ability to form glycoprotein analogues with the free saccharides.

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